

Abstracts

## 12th Annual Scientific Meeting of the Irish Society of Human Genetics, Friday 18th September 2009.



Nursing Building, Dublin City University, Ireland.

### PROGRAMME:

10.00 – 11.00	Registration / Tea and Coffee
11.00 – 11.05	Welcome
11.05 – 12.00	Plenary I: Clinical Research - 4 Spoken Presentations:
12.00 – 13.00	<b>Keynote address:</b> "Ophthalmic-acromelic syndromes in mouse and man" <b>Dr David Fitzpatrick</b> , MRC Human Genetics Unit, Western General Hospital, Edinburgh
13.00 – 14.00	Lunch and Poster viewing
13.45 – 14.00	Council Meeting
14.00 – 15.30	Plenary II: Basic Research - 6 Spoken Presentations:
15.30 – 16.00	Tea and coffee / Poster viewing
16.00 – 16.15	Business Meeting
16.15 – 17.15	<b>Keynote address:</b> "Mapping complex traits - The human and canine genetic systems" <b>Dr Elaine Ostrander</b> National Human Genome Research Institute, NIH, USA
17.15 – 18.00	Wine reception / Presentation of Prizes / Meeting Close

### SPOKEN PAPERS:

#### S01. Cytogenetic Analysis in Donor Cell Neoplasms.

Johanna Kelly<sup>1</sup>, Natasha Coen<sup>1</sup>, Lynn Barton<sup>1</sup>, Michael O'Dwyer<sup>2</sup>, Paul Browne<sup>3</sup>, Eibhlin Conneally<sup>3</sup>, David R. Betts<sup>1</sup>

<sup>1</sup>National Centre of Medical Genetics, Our Lady's Children's Hospital, Dublin, Ireland, <sup>2</sup>Department of Haematology, University College Hospital, Galway, Ireland, <sup>3</sup>Department of Haematology and Oncology, St. James's Hospital, Dublin, Ireland.

Donor cell neoplasms (DCN) are a rare entity, and the vast majority reported are either AML or ALL. We report two new cases (males, aged 25 and 43) that had an allogeneic SCT from female related donors in first CR following an initial diagnosis of AML and ALL and respectively. Both patients, approximately 5 years following transplant, re-presented with neoplastic disease which was shown to be of donor cell origin by cytogenetic methods. For patient 1, the AML showed an apparently normal karyotype. Following the occurrence of new myeloid-lineage related irregularities, a bone marrow aspirate displayed features of MDS/CMML. Cytogenetic

analysis revealed a 45,XX,-7 karyotype, thereby proving the donor cell origin of disease. Cytogenetic analysis was not performed on the ALL of patient 2. On re-presentation with lymphadenopathy the bone marrow morphology was consistent with a diagnosis of DLBCL. Conventional cytogenetics was not possible, however, FISH analyses showed a MYC rearrangement and all cells had an XX sex chromosome complement. Both these patients are unusual in that their diseases have been seldom reported as donor cell neoplasms. This study demonstrates that in patients with a possible disease relapse following an allogeneic SCT a DCN also needs to be considered.

**S02. Determination of the contribution of H63D/H63D genotype to iron overload, and validation of a dual hybridisation probe assay for detecting HFE genes.**

Kathy Nolan, Mark Dobson, Joanne Brady, Christine Brady, David Barton.

National Centre for Medical Genetics, OLCH, Crumlin.

Hereditary haemochromatosis (HH), a disorder of iron metabolism, is caused by mutations in the HFE gene. Most patients are homozygous for the C282Y mutation, or compound heterozygotes for C282Y and H63D. The contribution of the H63D/H63D genotype to iron overload is not well characterised. We determined the prevalence of this genotype in 520 query affected HH patients (presenting with transferrin saturation >45%) in order to measure the contribution of this genotype to iron overload in the Irish population. Results were compared to the prevalence in 520 blood donors, as HH patients were excluded from donating blood in Republic of Ireland at the time of collection. We found that the H63D/H63D genotype was significantly over-represented in the iron overload group. The allele frequencies for all HFE mutations were found to be higher than previous estimates, indicating that only 51% of the Irish population have a normal genotype at this locus. A novel HH assay for the detection of HFE mutations using HybProbes on the Roche LightCycler was validated as part of the study. The assay was found to be 100% sensitive, specific, robust, repeatable and reproducible.

**S03. Familial Learning Disability and dysmorphism due to a cryptic insertional translocation determined by CGH array.**

Patricia Foley, Rosemarie Kelly, Nicole de Leeuw\*, Andrew Green.

NCMG, Our Lady's Children's Hospital, Crumlin, Dublin 12, \*Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands.

We describe a family of six individuals with severe unexplained

learning disability and dysmorphism in two generations who had been repeatedly investigated over a 15 year period, with no abnormality found. A cryptic chromosomal rearrangement had always been highly suspected but not proven until CGH array analysis. Affected family members showed similar facies of bitemporal narrowing, prominent jaw and abnormal palmar creases.

Array CGH analysis (whole genome; 32,000 clones with an average resolution of > 300 kb; UCSC genome browser May 2004 assembly) revealed an interstitial, 2.1 Mb loss in 2q36.3q37.1 (230.1-232.2Mb; 26 BACs, 18 genes) in three affected family members. The unaffected parent of each person carried a cryptic balanced 1:2 insertional translocation of 2.1 Mb of 2q36.3q37.1 into 1p13. Carrier testing has been carried out in nine healthy family members, and in one an asymptomatic duplication of 2q36.3q37.1 was noted.

We show how CGH array has 1. re opened a diagnosis for many families previously left without a cause for their disability 2. once a diagnosis is achieved allows carrier testing for asymptomatic family members 3. gives further insight as to the phenotypic variability of chromosome duplications.

#### S04. Mutational analysis of *COL8A2* in keratoconus and posterior polymorphous corneal dystrophy.

DP Dash, J Church, E Héon, CE Willoughby.

Queen's University of Belfast, Royal Victoria Hospital, Belfast and The Hospital for Sick Children Toronto.

**Purpose:** Mutations in collagen, type VIII, alpha-2 (*COL8A2*; MIM#120252) have been reported in posterior polymorphous corneal dystrophy (PPCD) and Fuch's endothelial corneal dystrophy (FECD). The role of *COL8A2* in PPCD and FECD remains controversial. Although PPCD and keratoconus (KCTN) involve different layers of the eye, PPCD has been associated with KC in several reports. The purpose of this study was to comprehensively screen *COL8A2* in PPCD and KCTN patients.

**Methods:** All patients had a full ophthalmic examination and the diagnosis of keratoconus and PPCD was made on the basis of clinical examination, a history of penetrating keratoplasty for keratoconus/PPCD and corneal topography. Mutational analysis of *COL8A2* was performed by direct cycle sequencing, in a multinational PPCD and KCTN patient cohort.

**Results:** A novel change, Ile171Val (c.514A>G) was detected in a PPCD patient. A previous reported pathogenic mutation in FECD, Arg155Gln (c.464G>A) was detected in a patient with KCTN from Canada. A previous reported pathogenic mutation in PPCD Thr502Met (c.1505C>T) was detected in a Canadian patient of Filipino ethnicity affected with PPCD. None of these variants were detected in Caucasian controls. Ile171Val was not seen in 24 black American controls. Arg155Gln and Thr502Met are reported in unaffected Japanese patients and we also found them in Filipino controls indicating racial polymorphisms.

**Conclusions:** Mutations in *COL8A2* play a minor role in the pathogenesis of posterior polymorphous corneal dystrophy and keratoconus, and are racially polymorphic.

#### S05. The Autism Genome Project: genome-wide association studies in autism.

Richard Anney, for the Autism Genome Project.

Department of Psychiatry, Trinity College Dublin.

Autism and autism spectrum disorders (ASD) are neurodevelopmental disorders that affect approximately 1 in 150 individuals and are characterized by deficits in reciprocal social interaction, communication and patterns of repetitive behaviours and restricted interests. Evidence to date supports high heritability and a complex genetic architecture. Thus far we have results from over 1500 ASD families, roughly half of the families we will analyze during this three-year phase of the AGP. This resource is sufficiently large to implement multiple analytical strategies for localizing susceptibility loci. We present data from association analysis from the additive model across three ancestry (all, Northern European and Southern European) partitions of the data split along two diagnostic group (any ASD) and a narrow diagnostic group (autism). At this point our results implicate only one gene from our main analyses, namely MACROD2. Other genes previously described in relation to autism have also been implicated by various splits of the data. The AGP is currently expanding the dataset to a replication analysis to 3000 ASD families. Whether these loci remain intriguing and whether new loci are implicated from this additional analysis will be determined by analyses to be presented at the 2009 ISHG.

#### S06. Mutation detection in 46 Retinitis Pigmentosa (RP) genes using targeted sequence capture and next generation sequencing.

Graeme R Clark, Dorota Muszynska, Sharon Alexander, Giuliana Silvestri, Colin E Willoughby, David A Simpson.

Centre for Vision and Vascular Science, Queen's University Belfast, Northern Ireland.

**Introduction:** Retinitis Pigmentosa (RP) is a clinically and genetically heterogeneous inherited retinal degenerative disease. Although the causative genes for approximately half of cases have been identified, it is currently difficult to test all of these and provide a genetic diagnosis for new patients. We have therefore developed a new high-throughput sequencing strategy to screen all known RP genes in a single assay.

**Methods:** A sequence capture array (Nimblegen) was designed which targets exons and splice sites of 46 known RP genes. Following enrichment of these regions from 5 RP patient DNA samples they were sequenced using a Genome Analyzer (Illumina). The ~10 million reads from each run were aligned to a reference sequence using Genomics Workbench software (CLCBio) and sequence variants detected.

**Results:** Analysis of the initial sequencing results confirmed the presence of a potentially pathogenic variant in RPGR in one sample. Many additional sequence variants, were identified, primarily known SNPs. The frequencies of novel variants are being assessed in a control population.

**Conclusion:** Targeted sequence capture followed by next generation sequencing provides an effective approach to the parallel screening of multiple genes, enabling the detection of both known and novel mutations in disease-associated genes.

#### S07. The use of SNP homozygosity mapping to identify disease genes in Irish families.

Jillian Casey<sup>1</sup>, Judith Conroy<sup>1</sup>, Regina Regan<sup>1</sup>, Naisha Shah<sup>1</sup>, Tiago Magalhaes<sup>2</sup>, Andrew Green<sup>3</sup>, Sally Ann Lynch<sup>3</sup>, Sean Ennis<sup>1</sup>.

<sup>1</sup>School of Medicine, University College Dublin, Ireland. <sup>2</sup>Instituto Gulbenkian de Ciencia, Oeiras, Portugal. <sup>3</sup>National Centre for Medical Genetics, Ireland.

Homozygosity mapping using high density SNP platforms has accelerated disease gene discovery in recent years. Compared to microsatellites, the resolution provided by SNP homozygosity mapping (SNP HM) offers a greater ability to fine-map the disease locus. We applied SNP HM to four disorders in consanguineous Irish families, 3 of which are Irish Travellers, an endogamous nomadic group. The disorders studied are microphthalmia (arCMIC), ACTH resistance (arACTHR), immunodysplasia (arIDS) and microcephaly. In the arCMIC study HM narrowed the genome-wide search down to four homozygous regions (0.9Mb) comprising 12 potential candidate genes. Of the 12 genes two emerge as strong functional candidates as both have been implicated in eye development. In one of the extended families with arACTHR we reduced the area of interest to 7 homozygous regions (1.38Mb with 9 genes). The arIDS study identified 33 candidate regions with 72 genes. In the microcephaly family, because of the level of homozygosity amongst the first cousin parents, we have only managed to reduce the candidate search to 188 genes. Further family samples from unaffected relatives should reduce the number of candidate genes to a manageable level. The candidate regions for arCMIC, arIDS and arACTHR are currently being sequenced in attempt to identify the disease-liable mutations.

**S08. Analysis of the function of spartin, a protein mutated in hereditary spastic paraplegia.**

Malgorzata Dytko, Paula Byrne.

School of Medicine and Medical Science, Conway Institute, University College Dublin, Ireland.

Hereditary spastic paraplegia describes a group of neurodegenerative diseases characterized by lower limb progressive weakness and spasticity. Troyer syndrome is an autosomal recessive form of hereditary spastic paraplegia caused by a frameshift mutation (1110delA) in the *SPG20* gene encoding spartin protein, the cellular function of which remains unknown. Knowledge about spartin interactors is also very limited. In this study we apply a broad spectrum of proteomics techniques to identify novel spartin binding proteins. We used a Tandem Affinity Purification technique followed by HPLC-mass spectrometry to characterize potential spartin binding partners. Selected putative interactions were confirmed by co-immunoprecipitation experiments. We identified 94 potential spartin-binding proteins which were grouped into functional categories. We performed co-immunoprecipitation experiments to confirm that spartin interacts with GRP78, GRP75 and nucleolin proteins. Additionally our mass spectrometry results confirmed previously published information about spartin interaction with ubiquitin and the E3 ubiquitin-protein ligases, AIP4/Itch and AIP5/WWP1. Our studies suggest that spartin is a multifunctional protein and for the first time we suggest a role for spartin in protein folding and turnover both in mitochondria and endoplasmic reticulum. We also show for the first time interaction between spartin and a nucleolar protein, nucleolin.

**S09. Parental Origin Bias in *de novo* CNVs Detected in Autism Probands.**

N Shah<sup>1</sup>, R Regan<sup>2</sup>, J Conroy<sup>2</sup>, T Magalhães<sup>4</sup>, J Casey<sup>2</sup>, R Anney<sup>5</sup>, A Green<sup>3,2</sup>, L Gallagher<sup>5</sup>, M Gill<sup>5</sup>, DC Shields<sup>1</sup>, A Vicente<sup>4</sup>, S Ennis<sup>2,3</sup>.

<sup>1</sup>UCD Conway Institute and UCD Complex and Adaptive Systems Laboratory, School of Medicine and Medical Science, University College Dublin, Dublin, Ireland, <sup>2</sup>Health Science, School of Medicine and Medical Science, University College Dublin, Dublin, Ireland, <sup>3</sup>National Centre for Medical Genetics, Our Lady's Hospital

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Genetic variation occurs in humans at both individual and population level. One such variation that contributes to human genetic diversity is copy number variation (CNV) of genomic segments. In addition to contributing to common variation among healthy individuals, CNVs are associated with a number of genetic disorders and the susceptibility to complex disorders. It has also been shown that there is an association between *de novo* CNVs and complex disorders including autism spectrum disorder (ASD). Such *de novo* mutations could occur in either maternal or paternal germ line or in developing embryos.

We are investigating whether there is a parental origin bias for *de novo* CNVs in 380 Irish and Portuguese ASD probands, which were genotyped on the Illumina 1M beadarray. In our preliminary analysis of *de novo* CNVs, we observed a bias towards paternal origin ( $P = 0.016$ ) for *de novo* deletions with 2 or more SNPs informative and congruent for defining parental origin from SNP genotype and intensity data (maternal: paternal origin ratio is 2:12 deletions). We also observed that there is a bias towards maternal origin ( $P = 0.041$ ) for *de novo* duplication CNVs (maternal: paternal origin ratio is 31:16 duplications).

Further studies on the relationship between duplication and deletion *de novo* CNVs and their parent of origin may provide further insights into the molecular mechanisms during meiosis. Replication of this study in a larger dataset and experimental validation is to be followed.

**S10. The MTHFR 677TT is Less Responsive to Folate and/or Riboflavin Deficiency Compared to the 677CC genotype as assessed by global gene expression changes.**

Linda Hughes<sup>1</sup>, Nicola Carroll<sup>1</sup>, Christian Fiedler<sup>1</sup>, Anne Parle-McDermott<sup>1</sup>.

<sup>1</sup>Nutritional Genomics Group, School of Biotechnology, Dublin City University, Dublin 9.

The functional consequences of the MTHFR 677C>T polymorphism are a thermolabile enzyme that releases its FAD cofactor more readily than wildtype, particularly in combination with low folate status. While the impact of this polymorphism to the enzyme has been well researched, an understanding of how low folate status increases risk of such a range of common human diseases and how the MTHFR 677TT genotype exacerbates this risk is far from complete. The aim of this project was to investigate the early genetic changes associated with the initial response of cells to folate/riboflavin deficiency *in vitro* using gene expression profiling and cell lines homozygous for each MTHFR 677C>T allele.

Coriell® lymphoblast cell lines homozygous for each allele, 17274 (TT) and 17158 (CC) were grown for 12 days in folate deficient, riboflavin deficient, folate/riboflavin deficient and control media. Cells were harvested for RNA and subsequently hybridised to Affymetrix HG U133 plus 2.0 GeneChips. Analysis of the normalised data showed that the MTHFR 677TT cells showed less gene expression changes than the 677CC cells under all nutrient deficient conditions. This indicates that the 677TT cells already harbour a 'folate/riboflavin deficient' expression profile. Novel folate/riboflavin responsive genes and pathways are being further investigated in both cell lines.



## POSTER PRESENTATIONS:

### P01. Familial transitional cell carcinoma of the bladder.

Deirdre E Donnelly, Robin Brown, Patrick J Morrison.

Department of Medical Genetics, Belfast City Hospital Trust, Belfast BT9 7AB, UK.

**Introduction:** Transitional cell bladder carcinoma is common. The only aetiological factors identified so far are cigarette smoking and certain occupational exposures. Familial cancer of the bladder is not widely documented. However, it has been reported that there is increased risk to relatives once a case has been diagnosed which does not appear to be related to familial clustering of smoking. Further research in this area may lead to identification of candidate genes, leading to better understanding of the pathogenesis of bladder carcinoma and, ultimately, improvements in treatment.

**Case history:** We present a family with three cases of transitional cell bladder carcinoma in the same sibship. The proband was diagnosed at the age of 73 years, his brother at 76 years and his sister at 60 years. There are two other sisters, one diagnosed with breast cancer at 66 years and one diagnosed with basal cell skin cancer at 80 years.

**Discussion:** We discuss the possible modes of inheritance in this family and the potential for identifying candidate genes. Screening recommendations for at risk relatives is also reviewed.

### P02. Familial Gall bladder carcinoma associated with malignant melanoma.

Patrick J Morrison, Deirdre E Donnelly.

Department of Medical Genetics, Belfast City Hospital, Belfast BT9 7AB.

**Introduction:** Gallbladder cancer is rare - two familial cases are reported in the literature and it is unclear whether a familial entity exists. We describe a family with familial cancer of the gallbladder and melanoma.

**Case History:** The index case had an adenocarcinoma of the gallbladder diagnosed at 64 years, and melanoma aged 43. Her brother had an adenocarcinoma of the gallbladder aged 61 and was noted to have a 'porcelain' gallbladder on resection. Another brother had gallstones aged 52. Her son had melanoma aged 34. Her mother had gallstones requiring cholecystectomy at age 50 and died of pancreatitis aged 71.

**Discussion:** The combination of gallstones and gallbladder cancer suggests a predisposing gene which may be autosomal dominant. The concurrent history of melanoma could suggest a link between melanoma and adenocarcinoma of the gallbladder. We review the genetics of cholangiocarcinoma and suggest screening recommendations for patients with a family history of gallstones and / or at least one cancer of the gallbladder with regular ultrasound and biochemical investigations.

**Conclusion:** Cancer of the gallbladder has a hereditary component and patients with a history of gallstones or cancer of the gallbladder should be questioned about family history, and if positive, screening instigated.

### P03. Replication of the finding that a SNP in the Human Renin gene enhancer region increases blood pressure.

C Vangjeli<sup>1</sup>, N Clarke<sup>1</sup>, U Quinn<sup>1</sup>, P Dicker<sup>2</sup>, O Tighe<sup>1</sup>, C Ho<sup>1</sup>, E O'Brien<sup>3</sup>, A Stanton<sup>1</sup>.

<sup>1</sup>Molecular & Cellular Therapeutics, Royal College of Surgeons in

Ireland, 123 St Stephens Green, Dublin 2, Ireland, <sup>2</sup>Department of Epidemiology & Public Health Medicine, Division of Population Health Sciences, Royal College of Surgeons in Ireland, Beaux Lane House, Mercer Street Lower, Dublin 2, Ireland, <sup>3</sup>ADAPT Centre, Beaumont Hospital, Dublin 9, Ireland.

Variants in key genes of the renin-angiotensin system have the potential to modulate blood pressure (BP). Using a tag SNP approach, we aimed to capture maximal genetic variation in the angiotensinogen, renin, angiotensin converting enzyme and angiotensin converting enzyme 2 genes. We tested for associations of these variants with BP in two Irish occupational cohorts. Twenty-four hour ambulatory as well as clinic BP was measured in population I and clinic BP in population II. Individual SNP, haplotype, step wise regression and two-way SNP interaction analysis were performed.

Of the 22 tSNPs, only the REN-5312C/T SNP showed consistently significant associations with elevated diastolic pressures. Carriage of one REN-5312T allele was associated with the following age and sex adjusted increments in diastolic pressures (mean [95% CI], mmHg); Population 1, clinic 1.5[0.3,2.8], daytime 1.4[0.4,2.4], night-time 1.3[0.4,2.3]. Population 2, clinic 1.1[0.1,2.1]. Haplotype analyses and multivariate analyses were in concordance with individual SNP analyses. The REN-5312T allele had previously been shown to result in increased in vitro expression of the renin gene. We have now shown, in two independent populations, that carriage of a REN-5312T allele is associated with elevated diastolic BP. Hence renin has been confirmed to be an important susceptibility gene for hypertension.

### P04. Not Presented

### P05. Tetrasomy 9p: a recognizable syndrome.

T Dabir, S McKee, S McCullough, L Rauch, G Smith.

Medical Genetics Department, Belfast City Hospital, Belfast, BT9 7AB, UK.

Tetrasomy 9p is a rare chromosomal aberration and was first described by Ghymers *et al.* in 1972. Since then less than fifty cases of tetrasomy 9p have been reported. Non-mosaic tetrasomy 9p cases have severe phenotype and poor prognosis. Previously described cases have a fairly recognizable phenotype comprising craniofacial, limb, uro-genital and cardiac abnormalities. We report 2 new cases of de novo non-mosaic tetrasomy 9p with similar clinical features.

**Case 1:** was diagnosed postnatally and survived for 10 days. She had dysmorphic features, large fontanelle, wide cranial sutures, hyper extended limbs with hip dislocations, Dandy-Walker malformation and congenital heart defects.

**Case 2:** was diagnosed antenatally and is currently 4 weeks old. The prenatal scan showed malrotated / hyper extended lower limbs with dislocated joints, complex congenital heart disease and Dandy-Walker malformation. She has similar dysmorphic features.

The most identifiable features of non-mosaic tetrasomy 9p seem to be characteristic craniofacial abnormalities, Dandy-Walker malformation, limb defects (hyper extended lower limbs with joint dislocations) and congenital heart defects. Antenatal diagnosis of tetrasomy 9p should be considered with these scan findings. The features noted in our cases and previously reported cases suggest that tetrasomy 9p is a recognizable syndrome with a distinct clinical phenotype.

**P06. Does inhibition of the Methylation cycle Impact on the same Genes as Folate and/or Riboflavin deficiency?**

Nicola Carroll, Linda Hughes, Anne Parle-McDermott.

Nutritional Genomics Group, School of Biotechnology, Dublin City University, Dublin 9.

Folate is an essential nutrient and suboptimal levels are associated with numerous common complex diseases. However, our understanding of the molecular mechanisms underlying these disease associations remains incomplete. Since one-carbon metabolism involves both the DNA and the methylation cycle, effects of folate deficiency could be exerted through disruption of either, or both, of these cycles. We sought to identify gene expression changes that occur following inhibition of the methylation cycle in an attempt to identify those genes/pathways that could play a role in folate-related disease risk.

A lymphoblast cell line was cultured in the presence of 10mM cycloleucine for 24 hours. RNA was then extracted from both control and treated cells, and microarray analysis was performed using the GeneChip® Human Genome U133 Plus 2.0 array in triplicate to identify differences in their gene expression profiles. A total of 91 genes were found to be up-regulated and 155 genes down-regulated in cycloleucine-treated cells following stringent statistical analysis ( $P < 0.01$ ). Pathway analysis of differentially expressed genes revealed a significant association with several biochemical pathways. We have focused on one pathway that also responded to folate deficient conditions. We are currently confirming this pathway association in additional nutrient deficient experiments.

**P07. PTEN - a family's story.**

Alex Magee.

Regional Genetics Service, Belfast City Hospital, Belfast BT9 7AB.

PTEN hamartoma tumour syndromes (PHTS) are rare, and include Cowden syndrome, Lhermitte-Duclos disease, Bannayan-Riley-Ruvalcaba syndrome and possibly Proteus syndrome. A common feature is cellular overgrowth leading to multisystem benign hamartomata.

This boy was referred at age 16m. Macrocephaly was suspected antenatally. He was born at 38wks, birth weight 4680gm. Hypotonia was immediately noted, as well as macrocephaly. Multiple investigations ensued. Development was delayed and parents had to cope with differential diagnoses of trisomy 21 and cerebral palsy. At consultation, it was noted that father was macrocephalic, and reported his own father was the same. PTEN was considered and a mutation confirmed – exon2 c.138C>A. At review, parents decided to pursue carrier testing and the mutation was confirmed in his father. They expressed concern about their older son. They felt he was macrocephalic (developmentally normal) and had been seen by ENT because of huge tonsils. On examination, it was considered likely that he too was affected and this was confirmed.

The couple hope to pursue PGD. They are aware that PTEN syndromes are underdiagnosed and feel that opportunities for clinical diagnosis were missed. They hope that their story will alert clinicians to the condition.

**P08. Natural History of a Case of Fucosidosis.**

Gillian Rea, Fiona Stewart.

Regional Genetics Service, Belfast City Hospital, Belfast BT9 7AB.

Fucosidosis is a rare Lysosomal Storage Disease. Inheritance is autosomal recessive. First described by Durand in 1966, there have been less than 100 reported cases. Although the ethnic origin shows a world-wide distribution, two populations with a relatively high incidence are Italians and the Mexican-Indian Population of New Mexico and Colorado in the USA. The severe deficiency of Alpha-L-Fucosidase leads to accumulation of fucose-containing glycol-lipids, glycol-proteins and oligosaccharides in various tissues. Features include (in descending order of frequency seen) progressive mental deterioration, progressive motor retardation, coarse facies, growth retardation, recurrent infections, dysostosis multiplex, angiokeratoma corporis diffusum, visceromegaly and seizures. Originally divided into type I (severe) and type II (mild), it is now recognised that there is a wide continuous clinical spectrum. There are 22 known mutations and all lead to nearly absent enzymatic activity. Suggesting clinical variability is not due to the nature of the mutation but to secondary unknown factors. Although the angiokeratoma corporis diffusum is a clinically useful hallmark of the condition, it may either be absent or not visible at the time of presentation. We present the case of a ten year old girl born to consanguineous parents who was diagnosed at two years of age.

**P09. Successful Treatment of Mucopolysaccharidosis Type II (Hunter Disease) With Idursulfase in a 36 year old man.**

FJ Stewart<sup>1</sup>, M McCloskey<sup>2</sup>, JE Wraith<sup>3</sup>.

<sup>1</sup>Belfast City Hospital, <sup>2</sup>Altnagelvin Hospital Londonderry, <sup>3</sup> Royal Manchester Children's Hospital.

Our patient was diagnosed with Mucopolysaccharidosis (MPS) Type II at the age of 12 years. MPS II is caused by a deficiency of iduronate sulphatase which is one of the lysosomal enzymes. Unwanted mucopolysaccharides are stored within various tissues in the body. Developmental outcome ranges from severe learning disability to normal intelligence as is seen in our patient. Our patient had short stature, characteristic facial features, joint contractures and marked hepatosplenomegaly. He developed worsening respiratory problems and at age 28 he was started on overnight CPAP. His FEV1 dropped from 0.98 to 0.46. He started on idursulfase (Elaprase®) in June 2007 at the age of 36. He was gradually able to reduce his CPAP and within a year this was discontinued. His saturations are now 99% in room air. His hepatosplenomegaly has reduced and his waist is four inches smaller. He has much more energy and was able to cook Christmas dinner unaided. His joint contractures are also improving slightly.

This case demonstrates that treatment of an older MPS II individual may lead to a significant improvement in their clinical condition and quality of life and that treatment should not be discounted on the grounds of age.

**P10. Tissue Specific Mosaicism of a der(18) in a Developmentally Delayed Boy.**

Linda McArdle<sup>1</sup>, Sally Ann Lynch<sup>1</sup>, Sean Ennis<sup>2</sup>, Thomas Morris<sup>1</sup>, David R Betts<sup>1</sup>.

<sup>1</sup> National Centre of Medical Genetics, Our Lady's Children's Hospital, Dublin, Ireland <sup>2</sup> School of Medicine and Medical Science, University College Dublin.

Chromosomal aberrations are frequently associated with developmental delay and in most cases they can be identified by

analysis of peripheral blood. However, in rare cases tissue specific mosaicism may exist. We report a boy who on initial examination showed colobomas of the optic discs, developmental delay and epilepsy. Cytogenetic analysis on peripheral blood was performed but showed a normal 46,XY karyotype. At 1-year-old there was a development of obesity and constriction rings on arms and legs were also noted. Based on these findings, FISH analysis was performed to exclude a microdeletion of the Smith Magenis region. A follow up examination at 18 months showed microcephaly, refractory epilepsy and a MRI showed delay in myelination. As both obesity and constriction rings are seen in pigmentary mosaicism, a skin biopsy was performed and chromosome analysis of fibroblasts showed the following karyotype:

46,XY,der(18)inv dup(18)(q11.2q21.2)del(18)(q21.2)[14]/46,XY[6]

We could thereby demonstrate a mosaic partial trisomy of 18q11.2-21.2 and partial monosomy for 18q21.2-qter. A subsequent FISH analysis on buccal mucosa smears indicated that approximately 85% of these cells contained the der(18). This patient did not demonstrate the typical clinical picture associated with duplications and deletions of chromosome 18. The milder phenotype may be attributable to tissue-limited mosaicism.

#### P11. Not Presented

#### P12. Investigating promoter hypermethylation of wnt signalling antagonists in prostate cancer.

AS Perry<sup>1</sup>, O Raheem<sup>3</sup>, AM Kennedy<sup>2</sup>, TM Murphy<sup>1</sup>, L Marignol<sup>1</sup>, L Sullivan<sup>1</sup>, B Loftus<sup>4</sup>, T Lynch<sup>3</sup>, M Lawler<sup>1,2</sup>.

<sup>1</sup>Academic Unit of Clinical and Molecular Oncology and <sup>2</sup>Department of Haematology, Institute of Molecular Medicine, St. James's Hospital and Trinity College Dublin; <sup>3</sup>Department of Urology, St. James's Hospital; <sup>4</sup>Department of Histopathology, AMNCH and Trinity College Dublin.

Wnt signalling activates cell proliferation and pro-survival genes through nuclear translocation of  $\beta$ -catenin. Secreted Frizzled-Related Proteins (SFRPs) block Wnt signalling, resulting in phosphorylation and degradation of  $\beta$ -catenin, and thus loss of expression of its target genes. Aberrant activation of Wnts is well documented in human cancers, including prostate. We are investigating promoter hypermethylation and associated epigenetic silencing of *SFRPs* in CaP.

Methylation of *SFRP1*, *SFRP2*, *SFRP4* and *SFRP5* was investigated in CaP cell lines and tissue samples of CaP (n=40), benign prostatic hyperplasia (BPH) (n=37), histologically normal prostate (n=39) and preinvasive high-grade prostatic intraepithelial neoplasia (HGPIN) (n=15). *SFRP* gene expression was evaluated using the Human Wnt Signaling TaqMan Low Density Array.

Methylation frequencies in CaP were 11.11% (*SFRP1*), 72% (*SFRP2*), 0% (*SFRP4*) and 30% (*SFRP5*). *In vitro* studies revealed *SFRP2* hypermethylation in CaP cell lines (LNCaP, DU145, PC-3 and 22Rv1). Significantly lower *SFRP2* methylation frequencies and quantitative levels were found in histologically normal prostate (10.52%; relative methylation score (RMS)=0.35), BPH (11.54%; RMS=0.05), and HGPIN (15.38%. RMS=1.39) compared with CaP (72%; RMS=56.64),  $P<0.0001$ . Methylation of *SFRP2* was not significantly associated with tumour grade ( $P=0.47$ ) or TNM classification ( $P=0.38$ ), indicating that it is widespread throughout all grades and stages of CaP. Quantitative RT-PCR confirmed

differential expression of *SFRPs* in CaP compared with benign prostate tissue.

We demonstrated that hypermethylation of *SFRP2* is a frequent event in the pathogenesis of CaP. Methylation of *SFRP2* may be a useful marker of CaP.

#### P13. Testing times: when the BRCA1/2 test comes back negative in families with a strong history of breast +/- ovarian cancer.

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Approximately 70% of families with a clear-cut autosomal dominant transmission of breast +/- ovarian cancer will carry a mutation in BRCA1 or BRCA2, but the pickup rate in most high-risk families is significantly lower than this. Many small families may still have a predisposing mutation, but lack a highly suggestive pedigree, whereas large families may display ascertainment bias. Many units now triage gene testing using the "Manchester Scoring System", which allots a score to each cancer in a lineage, and only those families meeting the scoring threshold are routinely tested. However, the DNA sample typically comes from a single affected family member; if the result comes back negative, this can mean a number of things. Firstly, the cancers may represent a chance cluster. Secondly, there may be a mutation (in BRCA1, BRCA2 or another gene) that cannot be detected by the test. Thirdly, there may still be a detectable gene in the wider family, but the person tested may have a coincidental cancer unrelated to the family mutation. Identifying families for further testing is important, and can be based on statistical analysis of the pedigree, enabling rational targeting of resources and maximisation of the chances of picking up clinically relevant mutations.

#### P14. Monozygous Twins discordant for Landau-Kleffner Syndrome.

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Landau-Kleffner syndrome (OMIM 245570), also known as a syndrome of acquired epileptic aphasia, was first described in 1957. The disorder is characterised by gradual or rapid loss of language in a previously normal child. All children have abnormal EEG compatible with the diagnosis of epilepsy; however, only 70% have clinical seizures. Additional features include behavioural disturbances (including on occasions features consistent with autism), cognitive regression of variable degree, and sometimes motor difficulties, highlighting the pervasive nature of the disorder. Males are more frequently affected than females (ratio 2:1). The aetiology for this syndrome remains unclear.

We report a case-study of monozygous female twins discordant for LKS. A previous MZ discordant twin pair with the same syndrome has been reported by Feekery *et al*<sup>1</sup>. In this case-report, one of the twins presented at 3 years of age with developmental concerns, regression of speech and epilepsy. Her unaffected twin continues to develop normally. This study examined the potential role of CNVs in the occurrence of LKS using the Illumina 1M Infinium SNP array. We also investigated the possible role of differential methylation, as a similar disease mechanism is observed in discordant MZ twins for Beckwith-Wiedemann syndrome. Results will be presented at this meeting.



<sup>1</sup>. Feekery CJ, Parry-Fielder B, Hopkins JJ. Landau-Kleffner syndrome: six patients including discordant monozygotic twins. *Pediatr Neurol* 1993;**9**(1):49-53.

**P15. Investigation of the NOS1 Ex1f VNTR and cognitive function in schizophrenia.**

Emma M Quinn, Sarah Furlong, Michael Gill, Aiden P Corvin, Gary Donohoe, Derek W Morris.

Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine and Dept. of Psychiatry, Trinity College Dublin, Ireland.

Schizophrenia is a chronic debilitating psychiatric disorder that affects approximately 0.5-1% of the population worldwide. The neuronal isoform of nitric oxide synthase (NOS1) gene has been identified as a putative susceptibility gene for schizophrenia based on previous linkage and association results and its glutamatergic functions within the brain. We previously reported an association between NOS1 and verbal IQ and working memory, two neurocognitive endophenotypes commonly studied in schizophrenia research. This finding was replicated in an independent German sample (Donohoe et al, *in press*). The NOS1 genetic variant in this study was a SNP (rs6490121) located in intron 10 of the gene that had no obvious impact on gene function. A highly polymorphic dinucleotide repeat termed NOS1 Ex1f VNTR is located in the NOS1 promoter. The short alleles of this VNTR are associated with decreased transcriptional activity of NOS1. Data from HapMap indicates that the risk G allele of rs6490121 is in linkage disequilibrium with the short alleles of NOS1 Ex1f VNTR. We investigated this putative functional mechanism of cognitive performance dysregulation at NOS1 by analyzing this VNTR in our schizophrenia case-control samples. The results of this study indicate that VNTR is not associated with verbal IQ and working memory but is associated tests of episodic memory. While working memory tasks are more sensitive to pre-frontal brain function, episodic memory is more reliant on temporal lobe function. The discrepancies in findings for working memory (associated with the NOS1 SNP rs6490121) and episodic memory (associated with the NOS1 VNTR) may relate to differences that reflect the more ubiquitous influence of NOS in brain function that is being differently emphasised across the two studies.

**P16. First patient with 16p11.2 submicroscopic deletion detected by array CGH in Northern Ireland Regional Genetics Service.**

Lisa Bradley, Simon McCullough, Peter McGrattan, Susan McNerlan, Geoff Smith, Mervyn Humphreys, Vivienne McConnell.

Northern Ireland Regional Genetics Service, Floor A, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB.

The 15 month old female proband is the first child of non-consanguineous parents born after a pregnancy using assisted conception. Complex congenital cardiac disease, dysmorphism, cleft palate, right strabismus, growth retardation and developmental delay, are the cardinal features observed in the proband. Echocardiogram showed complete AVSD, supracardiac TAPVD and PDA.

The following genetic investigations were normal: karyotype, 22q11and 9q34.3 FISH, subtelomere (P036D) MLPA screen, and microdeletion/duplication (P245A2) MLPA screen. Both parents showed dysmorphic features. The 41 year old mother also had learning difficulties and short stature, while the 34 year old father had two brothers with learning difficulties and epilepsy and reported

a great-uncle dying at 3 days old of an unknown cardiac condition.

Array CGH analysis using the Agilent Oligo 4x44k platform detected an approximate 0.5Mb deletion within the short arm of chromosome 16, region 16p11.2, from base pair 29581456 to base pair 30106254. This ~0.5 Mb deletion was confirmed using the Illumina HumanCytoSNP-12 platform and microdeletion/duplication (P297B1) MLPA screen.

Both parental karyotypes and microdeletion/duplication (P245A2 and P297B1) MLPA screens were normal, excluding an inherited abnormality. Parental array based CGH results are pending.

The case presented is the first positive microarray analysis result from the NIRGS following the recent introduction of array CGH to our repertoire of clinical genetic investigations.

**P17. The application of Multiplex ligation-dependent probe amplification (MLPA) for investigation of pregnancy loss.**

Simon McCullough<sup>1</sup>, Niall Kissick<sup>2</sup>, Geoff Smith<sup>1</sup>, Mervyn Humphreys<sup>1</sup>.

<sup>1</sup>Northern Ireland Regional Genetics Service, Belfast City Hospital, Belfast. <sup>2</sup>Department of Biochemistry, Queens University, Belfast.

Approximately 15-20% of clinically recognised pregnancies end in spontaneous abortion. Around 50% of these losses will have abnormal karyotypes following chromosome analysis. The most common chromosomal abnormalities in first trimester abortuses are autosomal trisomies, monosomy X and triploidy. One in 500 individuals are carriers of balanced translocations and these individuals have a higher incidence of pregnancy loss due to unbalanced segregants at meiosis. Chromosome analysis is therefore an important test in the investigation of pregnancy loss. This not only provides a reason for the loss of the pregnancy but also identifies those couples who require follow up karyotyping or amniocentesis in future pregnancies. Culturing of tissue samples from spontaneous abortuses often has a low success rate due to the quality of sample received and their delay in transit to the laboratory. The MLPA technique is performed on DNA and therefore does not require cells to be cultured. We have used subtelomere MLPA to investigate tissue samples received from spontaneous abortions and intra-uterine deaths. We will show that this technique has a high success rate compared with karyotyping and is reliable in the detection of chromosomal aneuploidy in tissue samples.

**P18. Evaluation of long range PCR methods for resequencing of schizophrenia candidate genes using next generation sequencing technology.**

Amy S Gates, Elaine M Kenny, Lynne E Cochrane, Colm T O'Dushlaine, Emma M Quinn, Michael Gill, Aiden P Corvin, Derek W Morris.

Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine and Dept. of Psychiatry, Trinity College Dublin, Ireland.

Schizophrenia is a devastating complex neuropsychiatric disorder, affecting approximately 1% of the population, with significant health, social and economic impact in Ireland. The disorder is highly heritability and may be caused by abnormal neurodevelopment. Recent genome-wide association studies (GWAS) have identified a number of large copy number variants, which appear to be highly penetrant risk factors for schizophrenia (ISC, 2008). This consortium has also identified a number of loci where common genetic variation is contributing to schizophrenia risk (ISC, in

press). A project underway in our group is to study schizophrenia candidate gene(s) regions identified by this GWAS and identify all common genetic variation at these loci and map true schizophrenia causal variant(s).

A pilot resequencing project was designed to resequence genomic locations of interest in a control population of HapMap samples using next generation DNA sequencing technology.

We found very high concordance between SNPs called from the sequences generated on the Illumina platform and known HapMap SNPs for each individual DNA sample.

This pilot study has shown that ultra-high throughput technology is extremely useful in the rapid sequencing of genes and can generate dense maps of genetic variation for study of common variants associated with disorders such as schizophrenia.

#### P19. Development of methods for resequencing of genes using indexed DNA samples and the SureSelect target enrichment system on the Illumina Genome Analyzer.

EM Kenny, AS Gates, LE Cochrane, M Gill, AP Corvin, DW Morris.

Neuropsychiatric Genetics Research Group, Institute of Molecular Medicine and Dept. of Psychiatry, Trinity College Dublin, Ireland.

Next-generation sequencing technology has allowed sequencing of whole genomes to be carried out in standard molecular genetics laboratories. However, an important application of this technology is sequencing of specific genomic regions, for example disease genes in patient samples. In order to sequence parts of the genome of interest, a number of methods have been developed including long range (LR) PCR and microarray capture. Agilent Technologies have developed the SureSelect Target Enrichment System which allows targeting of 3.3Mb of the genome by using cRNA baits. Indexing methods have also been developed for next generation sequencing that allow multiplexing of samples in one sequencing library. We have combined the SureSelect Target Enrichment System with an indexing protocol to develop a cost-efficient method for targeting smaller regions of the genome in multiple DNA samples. We evaluated this method by comparing the sequence data produced using DNA enriched with the SureSelect system to data generated by a LR PCR enrichment of the same target sequence.

We will present data on the performance of the SureSelect method in comparison to the LR PCR method in HapMap test samples and present a protocol that will be extremely useful in the rapid sequencing of target genomic regions.

#### P20. Spectrum and incidence of BRCA1 and BRCA2 mutations in the Republic of Ireland – An Audit.

Trudi McDevitt<sup>1,2</sup>, Mary Higgins<sup>1,2</sup>, Anne Crowley<sup>1,2</sup>, Nuala Cody<sup>1,2</sup>, Marie Meany, Cliona de Baroid, Maureen Adams<sup>1,2</sup>, Carmel Nolan<sup>3</sup>, Michael Farrell<sup>3</sup>, Eileen Berkeley<sup>3</sup>, Roisin Clarke<sup>3</sup>, Peter Daly<sup>3</sup>, Andrew Green<sup>1,2</sup>, David Barton<sup>1,2</sup>.

<sup>1</sup>National Centre for Medical Genetics, OLCHC, Crumlin, Dublin; <sup>2</sup>University College Dublin; <sup>3</sup>HOPE Directorate, Haematology, Oncology and Palliative Care Service, St James's Hospital, Dublin 8.

Comprehensive mutation screening of BRCA1 and BRCA2 has been available to Irish breast cancer families since 2005 via our Centre. We present an audit of the data to date. In total, pathogenic

mutations have been identified in 154/462 families (33%). The spectrum of these mutations comprises nonsense (BRCA1: 21, BRCA2: 3), frameshift (BRCA1: 30, BRCA2: 56), splice-site (BRCA1: 7, BRCA2: 2), substitution (BRCA1: 5, BRCA2: 6) and large deletions (BRCA1: 22, BRCA2: 2). Overall, the incidence of large deletions was found to be approximately 5% in the patient group screened to date, accounting for approximately 15% of the total mutation incidence, and appears to be higher than that reported by other populations to date. Eight mutations have been identified in more than 3 apparently unrelated families: BRCA1: p.E143X (19), c.1294\_1333del40 (7), exon 3 deletion (4), exon 21-24 deletion (4); BRCA2: c.8525delC (9), c.983del4 (6), c.2117delC (7). We present preliminary haplotype data for one of these recurrent mutations, which supports the presence of a possible founder effect. In addition, a large deletion encompassing exons 1-23 of BRCA1 has been identified in 4 families. We present microarray data which suggests that this may be an identical rare deletion in 4 apparently unrelated families.

#### P21. Association between polymorphisms in the gene regulated by estrogen in breast cancer 1 and bone mineral density variation in Caucasians.

KG Hegarty<sup>1</sup>, M Daly<sup>1</sup>, S Chavrimootoo<sup>1</sup>, F Shanahan<sup>3</sup>, MG Molloy<sup>2</sup>.

<sup>1</sup>Medicine, <sup>2</sup>Rheumatology and Sports Medicine, <sup>3</sup>Alimentary Pharmabiotic Centre, National University of Ireland, Cork, Ireland.

Using informative SNPs, we performed a gene-wide association study between a positional candidate gene, the gene regulated by estrogen in breast cancer 1 (*GREB1*), located at 2p25 and variation in bone mineral density (BMD) at the lumbar spine (LS) and the femoral neck (FN). Single marker and haplotype-based association testing was performed in a family-based discovery cohort (n = 508 (n = 229 families)) and a postmenopausal population-based replication cohort (n = 477). Significant total- and within-family association was observed between GREB1\_03 (A/G; MAF 0.09) and variation in FN BMD ( $P = 0.003$  and  $0.004$ , respectively). There was significant within-family association observed between GREB1\_03 and LS BMD variation ( $P = 0.005$ ). In the replication cohort, GREB1\_03 was not significantly associated with variation in BMD at either skeletal site ( $P > 0.05$ ). Another GREB1 polymorphism, GREB1\_04 (A/C; MAF 0.38), was significantly associated with FN BMD ( $P = 0.005$ ). GREB1\_04 is located in the same HapMap LD block as GREB1\_03. *In-silico* analysis suggests that the associated markers may affect *GREB1* enhancer binding and splicing regulation. These results suggest that variation within *GREB1* may contribute to BMD variation. Replication in larger, independent studies is required before functional analysis is undertaken.

#### P22. The Northern Ireland Tuberous Sclerosis Complex Database - TSC1 & TSC2 mutations in the tuberous sclerosis complex population in Northern Ireland.

Hilda Crawford, Charles Shepherd, Shane McKee, Patrick J Morrison.

Northern Ireland Genetics Centre, Belfast Trust, A Floor, Belfast City Hospital, Lisburn Road, Belfast BT9 7AB.

**Introduction:** There is almost complete ascertainment of the TSC population in Northern Ireland with 90 living and 8 deceased patients in 72 families in a general population of 1.68 million. The Northern Ireland Tuberous Sclerosis clinic offers a clinical genetic



service to TSC families. Mutation analysis is routinely offered to affected patients.

**Results:** In our database, 15 individuals in 8 families have a mutation in TSC1 and 34 individuals in 23 families have a mutation in TSC2. In 1 TSC2 family, a mutation negative parent of an affected child is presumed mosaic. In 6 families, no mutation was found. In 1 family, there is a presumed non pathogenic mutation in TSC2. In 3 families, results are pending. In the remaining patients, mutation analysis has not been done.

**Conclusion:** Identification of mutations in TSC confirms diagnosis and offers genetic testing to family members. It offers the possibility of prenatal genetic diagnosis for families who wish to exercise this choice. For clinicians, it offers a greater understanding of the condition and the possibility of genotype phenotype correlation.

**P23. The incidence of Fragile X syndrome in Northern Ireland, 2000-2006.**

Deirdre E Donnelly, Alex C Magee, Patrick J Morrison.

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**Introduction:** Fragile X is thought to be one of the commonest genetic causes of mental retardation. Affected individuals can also have autistic/behavioural problems, speech delay and seizures. On examination, patients have a large head with prominent forehead and large ears. Fragile X is X-linked and is much commoner, and more severe, in males. It is caused by a triplet repeat expansion on the X chromosome and carrier/pre-mutation repeats can be found in parents.

**Audit:** We examined our database for cases of Fragile X syndrome diagnosed between 1st Jan 2000 and 31<sup>st</sup> December 2006. We also identified patients diagnosed with Fragile X syndrome who were born in this 6 year period. Patient data was compared to find the commonest presenting features.

**Discussion:** In total, 23 were diagnosed and 3 were born within the study period. This gives an incidence figure of 0.0028 per 10,000 live births per year. This figure is much lower than would have been expected and we suspect that there may be a degree of under-diagnosis in our population. We would like to raise awareness of Fragile X and its common clinical features.

**P24. Incidence of Fragile X syndrome in the Republic of Ireland.**

<sup>1</sup>Michael Sweeney, <sup>2</sup>L Baker, <sup>3</sup>CA Graham, <sup>1</sup>DE Barton, <sup>1,2</sup>SA Lynch.

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The incidence of Fragile X varies from 1 in 2300 to 1 in 6,000 and there's evidence of geographical variation, with Taiwan having a low incidence (~ 1 in 10,000) compared to an incidence of ~1:2500 in Spanish newborns. Here we report the incidence of Fragile X cases identified at the NCMG and the Northern Ireland Regional Genetics Centre. There were 423,983 live-births in the Republic of Ireland (ROI) between 2000-2006 and 16 new cases of Fragile X were identified in that period. However, only 5/16 Fragile X cases were born between these years (2 cases were identified in 2007 &

2008). This is a minimum incidence of 0.17 per 10,000 births [1 in 60,569 births]. As many hospitals on the Western seaboard send samples to genetic testing centres outside of the ROI, recalculation of the data using births for the Eastern seaboard (282,000) gives an incidence of 0.21 per 10,000 (1 in 47,619). Despite that Fragile X is a common request referral to NCMG, the overall pick-up rate (approx 0.3%) is low and the incidence of Fragile X is very low compared to other countries. It is possible that testing may not have been targeted at the right patient group or that many cases have yet to be identified (i.e. children born in 2005 and 2006 have yet to 'worked up' by the paediatricians). The absence of a founder effect in the ROI or the possibility that the incidence is much higher in Irish counties where samples have traditionally been sent elsewhere may also account for the low incidence reported here.

**P25. Maternal UPD 16 and low level mosaic trisomy 16 observed in Amniotic Fluid following non-mosaic trisomy 16 in CVS.**

Claire J Breen<sup>1</sup>, Bronagh O'hC<sup>1</sup>, Marice Mullarkey<sup>1</sup>, Aiveen Carey<sup>1</sup>, Rosie O'Shea<sup>1</sup>, Andrew Green<sup>1</sup>, David E. Barton<sup>1</sup>, Fergal Malone<sup>2</sup>, and David R. Betts<sup>1</sup>.

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Trisomy 16 is the most commonly observed trisomy in first trimester miscarriages, accounting for over 30% of autosomal trisomies. Mosaic trisomy 16 has rarely been reported at second trimester amniocentesis and it is even more exceptional for it to be seen in liveborns. We report a case of trisomy 16 observed in a CVS taken at 13 weeks gestation referred for a high triple test score. A subsequent amniocentesis taken at 17 weeks revealed a karyotype of 46,XX in all 30 cells analysed. UPD 16 studies were also carried out on cultured cells from the amniocentesis with a range of polymorphic chromosome 16 markers. This showed maternal UPD 16 and a low level, paternal in origin, trisomy 16. Retrospective interphase FISH analysis with an Abbott 16 centromere probe showed approximately 10% mosaic trisomy 16. It is most likely that the fetal UPD 16 arose as the consequence of a 'correction' or 'trisomy rescue attempt' of an initially trisomic conceptus, which in turn was due to a maternal nondisjunction event. It is recommended that amniocentesis, UPD studies, and detailed ultrasound examinations should follow detection of trisomy 16 observed at CVS.

**P26. Not Presented**

**P27. The copy number variant *LCE3C*/*LCE3B*-del on chromosome 1 confers susceptibility to psoriasis in the Irish population.**

AW Ryan<sup>1</sup>, E Linehan<sup>1</sup>, G Turner<sup>1</sup>, P Gallagher<sup>2</sup>, A Irvine<sup>1</sup>, O Fitzgerald<sup>2</sup>, B Kirby<sup>2</sup>, R McManus<sup>1</sup>.

<sup>1</sup>Department of Clinical Medicine, Institute of Molecular Medicine, Trinity College, Dublin, Ireland.

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Psoriasis (OMIM#177900) is an inflammatory condition of the skin and joints, which typically manifests as areas of inflammation and abnormal skin growth. Prevalence in European populations is approximately 1-2%. Both genetic (including HLA) and non-genetic (stress, streptococcal infection) factors are believed to play a role in susceptibility. A common copy number variant (CNV) involving the deletion of the *LCE3C* and *LCE3B* genes in the late cornified envelope gene cluster (1q21.3), which encode skin barrier proteins, has recently been associated with susceptibility to psoriasis

in several European populations. We have genotyped this deletion and 3 physically associated single nucleotide polymorphisms (SNPs, rs17659389, rs10888502, rs4112788) in 441 Irish psoriasis patients and 983 Irish controls. In addition, we genotyped 3 SNPs (rs1062470, rs130079, rs130076) in the known HLA associated region. There was strong association with all HLA SNPs ( $P < 10^{-6}$ ). As noted in previous studies, *LCE3C\_LCE3B-del* was in complete linkage disequilibrium with the *rs4112788-T* allele. Haplotype analysis showed an association between the *rs4112788-T/LCE3C\_LCE3B-del* haplotype and disease ( $P = 0.02$ ). Our results, therefore, confirm observations from other European populations. Interestingly, the observed frequency of *LCE3C\_LCE3B-del* in Ireland is among the highest observed, to date, with 49% of Irish people homozygous for the deletion.

#### P28. The European Molecular Genetics Quality Network (EMQN).

Outi Kämäräinen<sup>1</sup>, Simon Patton<sup>1</sup>, David Barton<sup>2</sup>, Rob Elles<sup>1</sup>.

<sup>1</sup>European Molecular Genetics Quality Network, c/o NGRL and Regional Molecular Genetics Service, St Mary's Hospital, Manchester, United Kingdom, <sup>2</sup>National Centre for Medical Genetics, Our Lady's Children's Hospital, Crumlin, Dublin 12.

Molecular Genetics testing forms an increasingly important part of the diagnostic process in all branches of medicine. Studies of the reliability of such testing have indicated a significant level of inaccuracy in laboratory reports, arising from errors in sample identification, genotyping or interpretation. The European Molecular Genetics Quality Network (EMQN) aims to raise and maintain the quality of Diagnostic Clinical Molecular Genetics Testing by providing external quality assessment (EQA) schemes. In 2008 EMQN provided 22 disease specific and 2 technique specific EQA schemes. The EMQN's schemes are organised by a panel of experts and DNA samples are sent to participating laboratories once a year. Participating laboratories are asked to perform their routine analysis and interpret the results. The reports are marked by a group of experts. The participants receive a report on their performance.

400 laboratories from 42 countries around the world participated in the EQA schemes in 2008 and over 1400 reports were evaluated from laboratories. The standards of genotyping accuracy were high but significant error rates were found and methods of reporting and interpreting data were varied. The error rate indicates a clear need for EQA to measure current standards of proficiency and encourage laboratories to raise their technical and reporting performance. EMQN is now the world's largest provider of EQA for genetic testing.

#### P29. Mapping homozygosity in Irish sporadic ALS patients to identify recessive susceptibility loci.

Russell L McLaughlin<sup>1</sup>, Simon Cronin<sup>2,3</sup>, David S Lynch<sup>2,3</sup>, Kim A Caulfield<sup>2,3</sup>, Daniel G Bradley<sup>1</sup>, Orla Hardiman<sup>1,3</sup>

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Studies have shown that extended runs of homozygosity (ROHs) across the genome are common in European populations. These may be evident in genome-wide single nucleotide polymorphism (SNP) datasets as an improbably high number of successive homozygous genotypes. Mapping the locations of ROHs common to a particular phenotype may reveal recessive trait loci. Using the computer programme PLINK, we have attempted to map ROHs in an Irish population and specify overlapping, allelically-matching ROHs common only to a subset of this population described phenotypically by a neurological condition called amyotrophic lateral sclerosis (ALS). Gene ontology analysis has shown an overrepresentation of neurologically important genes in the ALS group compared to controls. Using this ROH mapping technique, various biologically plausible candidate regions across a number of chromosomes have been revealed as potential recessive disease loci. Deep re-sequencing of these regions may indicate the locations of recessive mutations conferring ALS susceptibility.

#### P30. Genomic Analysis of Single Nucleotide Polymorphisms (SNPs) among Children and Adults Treated for Acute Lymphoblastic Leukaemia: A Single Centre Study.

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School of Nursing, Dublin City University, Glasnevin, Dublin<sup>1</sup>, Belfast City Hospital, Dept of Haematology, Lisburn Road, Belfast BT9 7AB<sup>2</sup>, Trinity College Dublin, School of Molecular Medicine, Dublin<sup>3</sup>.

**Aim:** The objective of the study was to explore if there was a relationship between overall survival and amplifications / deletions of diagnostic genes in adults and children treated on protocols UKALL XII and UKALL 2003. Adults (n = 15) treated on the UKALL XII protocol, (males n = 8 53.3%, females n = 7 46.7%), age range 15-67 yrs. Children (n = 12) treated on the UKALL 2003 protocol (males n = 7 58.3%, females n = 5 41.7%), age range 7 months – 16 yrs.

**Methods:** Bone marrow / peripheral blood samples were obtained at diagnosis. DNA was applied to Affymetrix Genechip Human Mapping Genome-Wide 5.0 SNP array.

**Results:** There were significant differences in overall survival times between patients who expressed or did not express amplifications on chromosome 9 ( $\chi^2 = 4.270$ , df = 1,  $p = 0.039$ ) with median survival times in those who did not express the amplification 5.7 months versus 21 months in those who did. N = 8 (89%) of children / adults who did not express amplifications on chromosome 9 died during this period. In those who did express amplifications on chromosome 9 n = 5 (31%) died.

Overall survival times for patients who expressed and did not express deletions on the CDKN2A gene approached significance ( $\chi^2 = 3.779$ , df = 1,  $p = 0.052$ ) with median survival times in those who expressed deletions 14 months and 20 months in those who did not.

**Conclusion:** Further exploration of these amplifications/deletions is indicated using larger sample sizes.